Ligand Mediated Sequestering of Integrins in Raft-Mimicking Lipid Mixtures: The Role of Bilayer Asymmetry and Cholesterol Content

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Lipid microdomains play an important functional role in plasma membranes. However, the small size and transient nature of lipid/membrane heterogeneities in the plasma membrane make characterization of microdomains and microdomain-related membrane processes quite challenging. To address this issue, we recently introduced a powerful model membrane system that allows the investigation of membrane protein sequestering and oligomerization in raft-mimicking lipid mixtures using combined confocal fluorescence spectroscopy, photon counting histogram (PCH), and epifluorescence microscopy. Our experiments on bilayer-spanning domains showed that $\alpha_v\beta_3$ and $\alpha_5\beta_1$ integrins predominantly exist as monomers and sequester preferentially to the liquid-disordered (l_d) phase in the absence of ligands. Notably, addition of vitronectin ($\alpha_v\beta_3$) and fibronectin ($\alpha_5\beta_1$) caused substantial translocations of integrins into the liquid-ordered (l_o) phase without altering receptor oligomerization state. Here we expand our previous studies and report on the sequestering and oligomerization state of $\alpha_v\beta_3$ and $\alpha_5\beta_1$ in asymmetric bilayer compositions containing coexisting l_o and l_d phases located exclusively in the top leaflet of the bilayer (bottom leaflet shows only l_d phase). Remarkably, in such a membrane environment, both integrins show a higher affinity for the top leaflet-restricted l_o domains in the absence of their respective ligands. A slight change in the integrin sequestration was observed after addition of their respective ligands. We also present experimental findings, which show that cholesterol content has a substantial influence on integrin sequestering and oligomerization in raft-mimicking lipid mixtures. The described experimental results highlight the potential importance of membrane asymmetry and lipid composition in the sequestering of membrane proteins in biological membranes.

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